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# Agricultural Research Service Progress Report

## The Russian Wheat Aphid
## Fourth Annual Report



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**Contents**

## Introduction

It has been almost six years since the introduction of the Russian wheat aphid (RWA) into the United States, and the insect continues to thrive as an economic pest of wheat, barley, and certain forage grasses. The latest economic data (Massey, 1991) indicates that during the growing season of 1989-90, RWA infestations resulted in 1.89 million insecticide-treated acres and an estimated 12.16 million bushel loss in grain production. Estimated cost of treatment was $15 million, and estimated value of grain loss was $33.4 million, for a combined loss of $48.4 million. Massey (1991) estimates the total losses for the years 1987 through 1990 to be $325.2 million for direct loss (control cost, yield loss, and grazing loss) and $332.9 million for indirect loss, for a total loss of $657.9 million.

This document is the fourth annual report of the research progress on RWA in the Agricultural Research Service of the USDA. The objective of the report is to briefly update readers on advances in technology that have taken place during the past year in this organization. However, many of these projects are cooperative with other organizations such as other ARS scientists and locations, state universities, experiment stations, and USDA-APHIS. These and other cooperative efforts by all of the agencies toward solving the RWA problem have been quite remarkable. These combined efforts greatly increase the possibility of successfully managing this pest in the future.

Space limitations for this report dictate brevity. If additional detail or information about the RWA or any of the projects listed is required, the researchers listed in the personnel section may be contacted directly.

## Personnel

New in RWA research is Richard Roehrdanz, Insect Genetist at Fargo, North Dakota, engaged in utilizing DNA-based genetic markers to identify strains of natural enemies of the RWA. Also, Lawrence Lacey, formerly working in the Azores on Japanese beetle, has joined the staff as Insect Pathologist at the European Biological Control Laboratory, Montpellier, France. Two new Research Associates have joined ARS: David Kazmer, to work on population biology of natural enemy introductions at Montpellier and Helen Belefant-Miller to work on plant physiology/insect interactions at Stillwater. Gary Puterka who worked on RWA genetics at Stillwater as a Research Associate has accepted a permanent position with the USDA, ARS Appalachian Fruit Research Station, Kearneysville, West Virginia.

Alternate Hosts for RWA

Stillwater, OK
Dean Kindler, Research Entomologist

Host Plant Resistance

Stillwater, OK
James Webster, Research Entomologist
Keith Mirkes, Agricultural Research Technician
Rusty Peterson, Senior Agriculturalist

Small Grains Germplasm Enhancement

Stillwater, OK
David Porter, Research Geneticist
Cheryl Baker, Geneticist
Dolores Mornhinweg, Assistant Researcher
Rita Veal, Biological Technician

RWA/Host Plant Interaction

Stillwater, OK
Robert Burton, Research Entomologist
John Burd, Biological Technician
Helen Belefant-Miller, Plant Physiologist

Brookings, SD
Robert Kieckhefer, Research Entomologist
Walter Riedell, Research Plant Physiologist

Insect Genetics

Stillwater, OK
Gary Puterka, Research Entomologist
John Burd, Biological Technician

Fargo, ND
Rick Roehrdanz, Research Geneticist

Biosystematics

Beltsville, MD
Manya Stoetzel, Research Entomologist

Simulation Modeling

Stillwater, OK
Norman Elliott, Research Biologist

Biological Control

Stillwater, OK
David Reed, Research Entomologist
Norman Elliott, Research Biologist
Brian Jones, Biological Technician
Wade French, Biological Technician

Montpellier, France
Keith Hopper, Research Entomologist
Lawrence Lacey, Insect Pathologist
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Brookings, SD
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Lawrence Ertle, Entomologist
Paul Schaefer, Research Entomologist
Ken Swan, Biological Technician
Joseph Tropp, Biological Technician

Ithaca, NY
Richard Humber, Microbiologist
Tad Poprawski, Research Insect Pathologist
Steve Wraight, Research Entomologist
Nancy Underwood, Biological Technician

## Locations

The European Parasite Laboratory, previously located at Behoust, France, has been consolidated with the former Rome Laboratory (Italy) to form the European Biological Control Laboratory. The new facility is located at Montpellier in the southern part of France, under the direction of Lloyd Knutson.

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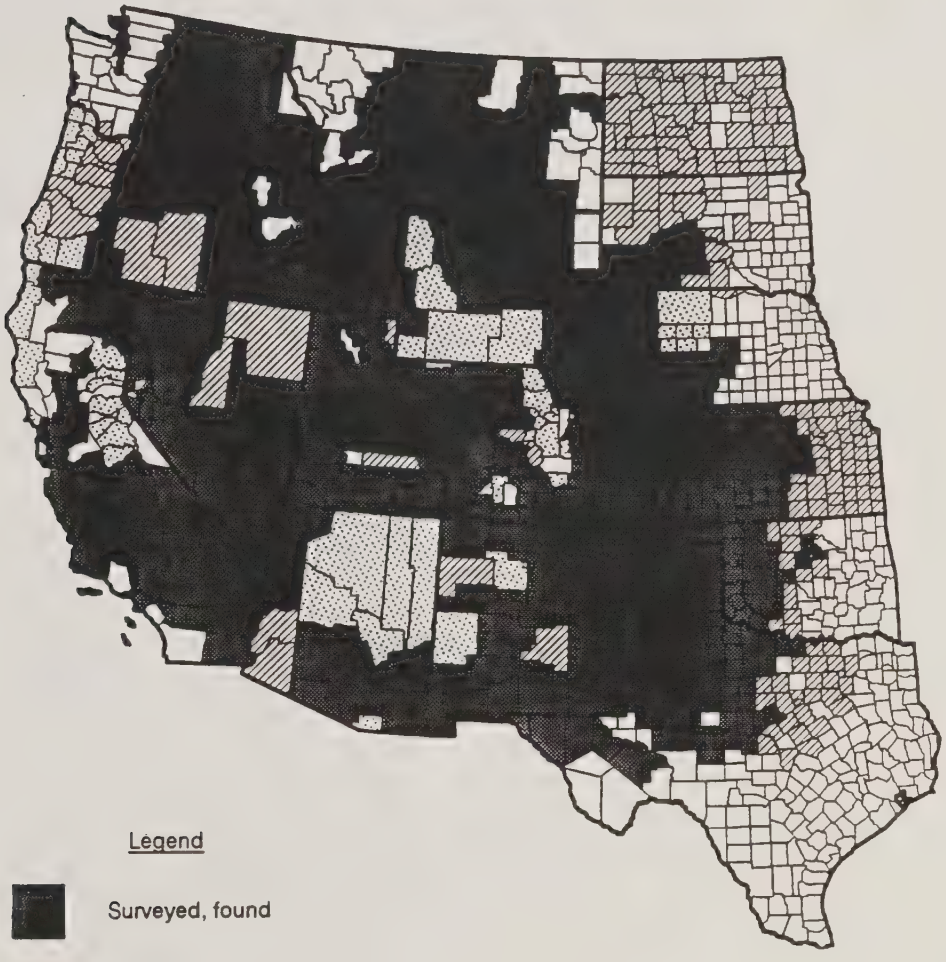
# RUSSIAN WHEAT APHID, 1986-1991



Legend

Surveyed, found

Surveyed, not found

No host crop

No data

Map by D. Cooksey
Montana State University
Department of Plant and Soil Science
November 7, 1991

Cooperative Agricultural Pest Survey (CAPS)
Map based on data from the National Agricultural Pest Information System (NAPIS)

## Alternate Hosts For Russian Wheat Aphid

**Mission:** To identify and characterize RWA-resistant germplasm lines that may serve as breeding resources for both cool- and warm-season cereals and turf, range, and conservation grass species.

**Plant Science Research Laboratory (PSRL)**
**Stillwater, OK**
**S. D. Kindler**

The Pacific Northwest is a major area of cool-season grass seed production. With the increasing importance of the RWA in the Pacific Northwest, there are concerns among seed industry members about the possible long-term effect that the RWA may have on cool-season grass seed production. In some cases, the aphid infestation has been controlled by the application of an insecticide. The objectives of our study are to determine which grass species are colonized and damaged by the aphid; to determine if genetic resistance occurs within genotypes of the same species; and to determine if fungal endophytes enhance aphid resistance in grasses.

Of the 15 grass species evaluated, aphid reproduction and survival was best on *Festuca* spp. when compared with the other species of grasses (excluding the checks). Nevertheless, all entries, regardless of species or cultivar, were affected by RWA as indicated by stunted growth and lower water content measurements. Under field conditions, the effects on the grass are rather subtle and would be identifiable only if checks free of RWA were maintained for comparison. If grasses were infested each year during the actual growing period and aphids increased to economic populations, it is conceivable that the effects could eventually interfere with carbohydrate transport to the roots and over a period of years could affect seed production directly and, eventually, cause a loss of plant stand.

Perennial ryegrass (*Lolium perenne* L. 'Repell' and 'Regal') had resistance 6 weeks after infestation. Redtop (*Agrostis alba* L. 'Streaker'), sheep fescue (*Festuca ovina* L. 'Azay'), slender creeping red fescue (*F. rubra* subsp. *litoralis* 'Logro'), and tall fescue (*F. arundinacea* Screb. 'Mustang', 'Apache', and 'Rebel') had intermediate resistance to RWA feeding. 'Repell' and 'Regal' were infected with a fungal endophyte, *Acremonium lolii* Latch, Christenson, & Samuels. 'Mustang' was infected with *Acremonium coenophialum* Morgan-Jones & Gams, and 'Wrangler' was infected with an unidentified species of *Acremonium*. Sheep fescue 'Bighorn' and strong creeping red fescue (*F. rubra* L. subsp. *rubra*, 'Ruby') were infected with *Epichloe typhina* (Pers. ex Fr.) Tul. 'Repell' and 'Regal' showed the greatest resistance to aphid feeding, suggesting that plant

resistance may have been enhanced by the presence of fungal endophytes.

By using isogenic lines of endophyte-free and -infected tall fescue and perennial ryegrass in another study, we determined that the presence of an *Acremonium* endophyte on RWA biology was much more dramatic on tall fescue than on perennial ryegrass. When RWA were confined to the base of leaves of tall fescue, the percentage survival of adults was not significantly different between endophyte-infected and endophyte-free tissue, but the percentage of nymphs surviving on endophyte-free tissue was significantly greater than on endophyte-infected tissue. The nymphs were much more sensitive to the presence of the endophyte than were the adults when confined to tall fescue. In contrast, the percentage survival of adults and nymphs on tissue of perennial ryegrass did not differ significantly between endophyte-infected and endophyte-free tissue. When given a choice, RWA preferred endophyte-free tall fescue leaf tissue and leaf sheaths for feeding sites by greater than a 6:1 margin over the same tissues infected by endophyte. In contrast, RWA showed no preference when given a choice between endophyte-free and endophyte-infected perennial ryegrass leaf tissue and leaf sheaths.

Except for a few species, the distribution and abundance of endophyte-infected grasses is poorly known. Further, the effects of fungal endophyte infection on insect herbivores has been examined for only a fraction of known host species. Therefore, researchers in the areas of plant resistance and the screening of grass germplasm for genetic resistance to RWA must be cognizant of fungal endophytes associated with grasses and their possible effects on RWA behavior and biology.

## Host Plant Resistance

**Mission:** To identify resistance sources, study the nature of this resistance, and cooperate with the Small Grain Germplasm Development program in the development and release of RWA-resistant small grain germplasm.

**Plant Science Research Laboratory (PSRL)**
**Stillwater, OK**
**J. A. Webster**

Germplasm Evaluation

The initial phase of a new host plant resistance program usually involves an extensive search for resistance. The RWA is no exception. Since its detection in the United States, we have evaluated over 35,000 small grains at least once for resistance to this pest. A summary of the collections tested since the beginning of the evaluation program is included in this report. Most of the material tested has come from the USDA-ARS National Small Grain Collection in Aberdeen, Idaho. Essentially all of the available rye and barley entries from this collection have now been tested for resistance to this pest; however, we will test new accessions of these species as they are cataloged and made available. Information about the resistance found in barley was reported in the Journal of Economic Entomology (Webster et al., 1991). Because the wheat collection is very large, consisting of about 60,000 accessions, we have tried to evaluate this material in a systematic approach, by evaluating entries from areas of the world believed to be the original home of the RWA, and also collections that appear to have considerable diversity. For example, at this writing, we are testing 1500 wheat lines from Ethiopia and China. We believe that this approach is the most productive. Information about other collections that may be good prospects for RWA resistance would be appreciated.

To maintain consistency during evaluations of the National Small Grain Collection, RWA from a colony originally established from RWA collected near Lubbock, Texas, in 1986 have been used. However, there is always the concern about new biotypes overcoming resistant lines, or the present colony losing its virulence. We currently maintain 33 RWA colonies from about 25 different locations within the United States. Preliminary virulence tests were conducted in the greenhouse with these colonies (and our original colony) on four wheat lines (PI 372127, PI 140207, CI 2401, and 'TAM W-101'), and on four barley lines (CI 1412, PI 366444, PI 366450, & 'Robust'). Differences in virulence between these colonies were not apparent when tested on these lines; however, more detailed tests are planned for the current year.

Most of the germplasm evaluation tests have been conducted in the greenhouse using the "hill" method, with 60 hills per greenhouse flat and five seeds of a test entry per hill. Test

seedlings are infested in the one-leaf stage with about 10 RWA per plant. Visual damage ratings are made at 15 and 25 days after infestation. Standard mixtures of greenhouse potting soil are used in the flats, and all tests are conducted under natural daylight. These procedures and the rating system have been outlined in previous reports, and in Webster et al. (1991). Although good resistance has been successfully detected in wheat, rye, triticale, and barley with existing procedures, there is much room for improvement, especially in the reliable identification of advanced generation plant material segregating for resistance. In some cases, it may also be desirable to identify accessions with lower levels of resistance, especially when resistant cultivars are to be used in conjunction with predators and parasitoids. Therefore, one of the ongoing projects of this unit is the development of improved germplasm evaluation techniques for RWA resistance. There are a multitude of variables involved in these tests, and each one of them needs to be critically examined.

Nature of Resistance

Information about the mechanisms of resistance (antibiosis, tolerance, and antixenosis) in RWA-resistant barley lines has been reported (Webster et al., 1991). Additional studies have been conducted recently using an electronic feeding monitor to compare RWA feeding behavior on resistant and susceptible barley lines. Results clearly show that the RWA confined to the resistant lines spend less time in phloem feeding activities than RWA confined to susceptible lines ('Wintermalt' and 'Morex'). This was especially true with RWA confined to the resistant line PI 366450 where very little feeding activity was exhibited. In addition, RWA that fed on the resistant barleys weighed less and were smaller. We are currently in the process of computerizing our electronic feeding monitors. With wheat, cooperative tests with the Germplasm Development Team showed that PI 140207, a white spring wheat originally collected in Iran, adversely impacted RWA fecundity, biomass, and developmental rates of RWA progeny produced on this line.

## Small Grain Germplasm Enhancement

**Mission:** To identify, characterize, and introgress genes conferring RWA resistance for small grain germplasm enhancement.

**Plant Science Research Laboratory (PSRL)**
**Stillwater, OK**
**D. R. Porter, C. A. Baker, D. W. Mornhinweg**

Wheat

From the many RWA-resistant hexaploid wheat accessions that have been identified to date, a core collection of 29 selections has been made. These selections have useful levels of RWA resistance, and some have acceptable agronomic traits. Each of the 29 selections in this group were hybridized with adapted RWA-susceptible wheat cultivars. F1 plants were grown in the greenhouse, and backcrosses were made to adapted RWA-susceptible cultivars; BC1 seed and selfed (F2) seed were harvested on an individual plant basis. F2 plants are currently being grown for production of F3 families. Genetic analysis of inheritance of RWA resistance will be done with both parents, F1, BC1, F2 populations, and F3 families.

In order to determine if these 29 selections carry different genes for RWA resistance, an allelism test was planned and crosses were made between as many lines as possible. F1 plants are being grown for production of F2 populations which will be screened for RWA resistance.

The mechanisms of resistance of PI 140207 were determined. Antibiosis and a moderate level of tolerance both appear to play a role in the resistance reaction. The size, weight, and developmental time of second generation aphids produced on PI 140207 were all detrimentally affected.

Uniform segregants within several F4 families derived from a cross between 'Bobwhite' and PI 149898 were identified and will be released as a source of RWA resistance for cultivar development.

Barley

Identification of *Hordeum vulgare* accessions with RWA resistance continued. All resistant accessions identified to date were retested in replicated trials. Of these accessions, 35 were found with good resistance (1 to 3 category of a 1 to 9 rating scale, 1 = healthy plant, 9 = dead plant), and 45 with moderate resistance (4 to 6 category). All 80 lines were evaluated agronomically in the field in Alberta, Canada, in the summer of 1991, and 44 were evaluated in the greenhouse at Stillwater during the spring of 1991. Also, four diploid *H. bulbosum* clones (CSU2, CSU12, 2920-1, and 2929-4) with good levels of RWA resistance were identified.

Crosses were made between the 44 resistant lines, and 'Morex' (a 6-row malting barley cultivar) and 'Crystal' (a 2-row malting barley cultivar). Backcrosses are planned for 1992. Resistant lines were also crossed to PI 366450 (a resistant accession), and a partial diallel was attempted among the 44 lines. F1 seed from 47 of these crosses is being increased to obtain F2 populations for genetic tests to determine the level of genetic diversity for RWA resistance in barley.

Two genetic studies were performed on parental, F1, and F2 seedlings of two crosses, 'Morex'/PI 366450 and 'Robust'/PI 366450. RWA-infested plants were rated for leaf streaking, rolling, and chlorosis. Plant growth measurements were also made on height, number of leaves, fresh and dry root and shoot weight, and total fresh and dry weight. Results suggest that a single dominant gene controls leaf rolling, with susceptibility being dominant. Leaf streaking also appears to be controlled by a single gene. Multiple gene control was indicated for chlorosis damage rating. F3 populations and backcrosses are being obtained for genetic tests which should clarify the inheritance of RWA resistance in PI 366450.

Cooperative work included screening of F2 and BC populations from wide crosses involving *H. vulgare* X *H. bulbosum*. Six F2 populations were screened for Busch Ag. Resources Inc., and resistant plants were saved for increase to the F3. F3 families are being screened from this material.

Cellular Resistance Studies

Developing improved RWA-resistant barley germplasm could be more efficient by understanding the physiological and biochemical mechanisms conferring whole-plant resistance. To gain insight, protein synthesis and accumulation during RWA feeding were monitored to determine cellular response to this stress. Preliminary work identified qualitative and quantitative protein differences when visualized by silver staining denatured leaf proteins resolved by isoelectric focusing polyacrylamide gel electrophoresis (IEF-PAGE). EIF-PAGE protein profiles of noninfested green seedling tissue of PI 366450 (resistant) and 'Morex' (susceptible) were virtually identical. However, when infested with 20 RWA per seedling plant at the 1.5-leaf stage, protein profiles of leaf tissue taken from the leaf tip (no direct RWA feeding sites were sampled) of PI 366450 differed from 'Morex'. Specifically, a complex of proteins (possibly three proteins focused close together) in the range of 22 to 24 kD disappear from the profile of 'Morex' after RWA infestation. Synthesis and accumulation of this protein complex continue in the resistant PI 366450, although the isoelectric point is shifted toward the basic end following RWA infestation. Identification of a variant cellular mechanism associated with the whole-plant resistance response in PI 366450 may prove beneficial as a marker for use in germplasm enhancement protocols.

## RWA-Host Plant Interaction

**Mission:** To develop a fundamental understanding of the molecular nature of the physiological and biochemical basis of RWA damage to facilitate the development of resistant germplasm derived from both traditional breeding programs and genetic engineering techniques.

**Plant Science Research Laboratory (PSRL)**
**Stillwater, OK**
**H. Belefant-Miller, D. R. Porter, J. D. Burd, R. L. Burton**

Barley plants under RWA infestation are obviously under stress and undergoing alterations in their water relations. The physiological responses of resistant barley plants may be different from susceptible plants. Knowledge of the resistance response may provide a powerful screening tool as well as insight into the overall mechanism of host plant resistance.

Toward this end, we are examining changes in various physiological parameters related to stress in response to RWA attack. Measurements are being made of stomatal conductance, relative water content, and abscisic acid levels of barley before and after RWA attack. Differences have been found between the responses of a susceptible barley cultivar, 'Morex', and a resistant germplasm, PI 366450. Preliminary results show that the resistant and susceptible barley plants have different amounts of abscisic acid and different levels of stomatal opening in response to RWA infestation. Relative water contents are similar in the leaves, but appear to be lower in the 'Morex' stem after infestation.

Several lines of evidence indicate that changes in the ultrastructure and photosynthetic function of the chloroplast occur under RWA infestation. Studies were conducted to ascertain whether measurements of chlorphyll fluorescence, on a total chlorophyll basis, would be of use as a rapid quantitative test for the early diagnosis of RWA damage and to evaluate its potential as a plant resistance marker among RWA hosts.

Overall, the fluorescence parameters measured were consistent with the associated plant response differentials previously determined from plant resistance screening studies. Consequently, measurements of chlorophyll fluorescence induction may provide a rapid and nondestructive method for evaluating plant damage that easily could be adapted to evaluate plants in greenhouse screening flats. Moreover, this technique may be especially useful in the absence of the visible chlorosis on which RWA damage ratings are generally based.

**Northern Grain Insects Research Laboratory (NGIRL)**
**Brookings, SD**
**W. E. Riedell, R. W. Kieckhefer**

Previous laboratory research conducted at the NGIRL indicated that grain yield loss to RWA was reduced by increased levels of fertilizer nitrogen (Riedell, 1990). If these effects could be repeated under field situations, nitrogen fertilization might be a useful strategy for limiting yield loss caused by aphids in plants that are deficient in this plant macronutrient (Riedell and Kieckhefer, 1992). This experiment was conducted to test this hypothesis under field conditions. A Brookings silty clay loam soil was planted in 'Arapahoe' winter wheat in the fall of 1990. Soil tests taken 10 April 1991 indicated that 70 lbs to the acre nitrogen was needed to attain a potential yield of 50 bu/acre. A split-plot arrangement of treatments, with 4 replications, with nitrogen fertilizer as main plots and RWA as subplots, was used. Liquid urea-ammonium nitrate (UAN, 28-0-0) was applied to the field in 7-foot strips at rates of 0, 35, or 70 lbs/acre on 21 April. On 7 May 1991, 1-$m^2$ cages were placed in the field, and half of the cages infested with RWA. Aphids were removed 17 days later. Analysis of plant samples taken from non-infested treatments on 29 May revealed that fertilizer treatment increased plant dry weight, tiller number, leaf area, and leaf chlorophyll content. At crop maturity, plants from five 1-foot segments of row were hand-harvested from each cage, and the grain threshed and cleaned. Statistical analysis appropriate for split-plot experimental design indicated significant effects of the fertilizer treatment ($F=9.5$, $DF=2$, $P=0.014$) as well as the aphid treatment ($F=27$, $DF=1$, $P=0.002$) upon grain yield. The fertilizer X aphid interaction was not significant. RWA infestation in plants given no nitrogen fertilizer resulted in a yield reduction of 40 percent , while the yield reduction of plants given 35 or 70 lbs nitrogen per acre were 29 or 25 percent, respectively. These field results indicate that application of recommended levels of nitrogen to winter wheat improves plant tolerance to RWA.

## Insect Genetics

**Mission:** To conduct national and worldwide biotypic and genetic studies on the RWA and its parasitoids.

**Plant Science Research Laboratory (PSRL)**
**Stillwater, OK**
**G. J. Puterka, J. D. Burd**

Worldwide Biotypic Variation Studies

Eight RWA isolates from a worldwide collection were evaluated on resistant and susceptible barley, triticale, and wheat entries. The aphid colonies originated from Seranon, France; Antibes, France; Disi, Jordan; Beyparazi, Turkey; Aleppo, Syria; Iachmen, Kirghizia; Komrat, Moldavia; and Lubbock, Texas, USA. Aphid population levels and damage ratings for leaf chlorosis, plant stunting, and leaf rolling were used to determine if biotypic variation occured within this species. Further, we determined what visible damage responses were best suited for detecting biotypes.

Overall, percentage leaf chlorosis was the best criteria for detecting biotypic variation in RWA on cereals. Yet, the differing mechanisms of resistance expressed by these cereals makes it important to consider other plant and insect factors. All plant entries responded differently to the RWA isolates, which indicated a high degree of biotypic diversity. Seven of the eight aphid isolates had unique virulence profiles. Moreover, isolates differed biotypically in countries where more than one isolate was collected (France and USSR)*. An isolate from the USSR was the most virulent, while an isolate from Turkey was the least virulent across all plant entries tested. Discriminant analysis showed that the USA isolate was most similar to a French isolate.

The entries that performed best against the RWA collection were the resistant triticales, PI 386148 and PI 386156, and the resistant barley, PI 366450. However, resistant plant germplasm will have geographical limits due to biotypic variation in RWA. In a positive sense, those cereal entries that are susceptible to RWA in one geographic area might actually be resistant in another geographic region. Thus, breeding for RWA resistance may not be necessary for certain geographic regions. All that may be needed is to identify those cereal cultivars that may already possess resistance to endemic RWA populations. In doing this, the need for an expensive breeding program could be circumvented (Puterka et al., 1992).

* Throughout this report USSR is used to refer collectively to the various republics in the former nation. At the time of collection of samples and of analysis, this reference was correct. For simplicity we have continued to use this designation when used by the various contributors to this report. However, it should be noted that the presence or absence of national boundaries has no bearing on findings reported.

Genetic Variation Studies

The genetics of RWA was investigated using starch gel electrophoresis of enzymes, sequencing regions of the mitochondrial DNA genome, and random amplified polymorphic DNA-polymerase chain reaction. RWA collections from wheat, barley, or other grasses were obtained from various countries throughout the world, with detailed sampling conducted in the United States. The collections included seven cloned colonies used in a study by Puterka et al. (1992) that were determined to exhibit biotypic variation, three clones from a study by Bush et al. (1989) that were shown to cause slight differences in damage to susceptible wheat, and a clone that was found to differ from other RWA in cuticular hydrocarbon profile.

Seventeen enzymes out of the 24 enzymes tested were found to be suitable for the genetic analysis. These 17 enzymes comprised 20 presumptive loci. Genetic variation in RWA was found in 3 of the 20 enzyme loci that were analyzed. However, genetic variation was only found among RWA populations from different countries. The alleles were fixed in populations within countries. Genetic differences were most notable in the Kirghizian, Jordanian, Moldavian, and Syrian clones, which also have been shown to be biotypically quite different from each other and the U.S. RWA population (Puterka et al., 1992). However, the genetic polymorphisms that were found did not serve as markers for the biotypic trait since the French, Turkish, and U.S. clones were also biotypically different, yet these clones had the same enzyme banding patterns. The phylogenetic analysis, based on three polymorphic loci for the collections consolidated into 12 countries, revealed that there were five distinct clusters. RWA from Moldavia, Kirghizia, and Ukraine were distinctly different from those from the other countries. The Kirghizian and the Kerson, Ukrainian RWA were more similar to each other than to RWA from other regions even though they are in close geographical proximity to the Crimea, Ukraine, Moldavia, and Turkey. South Africa, United States, Mexico, and Canada constituted one group of identical isozyme profiles, and these countries have RWA populations that were recently introduced. Enzyme electorphoresis did not detect enough genetic variation to determine the exact origin of the U.S. RWA population.

A new molecular genetic technique, RAPD-PCR, first reported by Williams et al. (1991), was successfully adapted for use on aphids to enable us to detect large genetic differences at the molecular level (Black et al., 1992). This technique uses the polymerase chain reaction (PCR) to amplify random regions of DNA (Random Amplified Polymorphic DNA) in an insect's chromosome. RAPD-PCR revealed genetic differences in four species of aphids--an insect group where genetic differences are rarely found using traditional genetic techniques. The aphids used included two collections of RWA. We determined that the South African RWA

population consisted of at least two different genotypes and that the Syrian population differed considerably from the South African population. This technique has also allowed us to detect two species of aphid parasites within the bodies of aphids. Our results indicate that RAPD-PCR will be useful in fingerprinting insect species, in the detection of parasitized insects, and in insect population studies. This method was used in conjunction with electrophoresis of enzymes for a detailed analysis of the RWA populations. Unique genetic banding profiles for RWA from each country were produced using RAPD-PCR. These data are still being analyzed.

**Biosciences Research Laboratory (BRL)**
**Fargo, ND**
**R. L. Roehrdanz**

Molecular studies were initiated to search for DNA-based genetic markers that could be used to identify laboratory colonies, geographically diverse populations, or closely related species of both parasites and predators of RWA. The work has focused on several species of parasitic Hymenoptera and predatory coccinellids. The need for reliable strain-specific and species-specific molecular markers cannot be underestimated. Their utility includes tracking the genes of a released strain, monitoring competitive studies between different strains, maintaining the genetic purity of lab colonies, identifying morphologically similar species, and assisting the biosystematics of complex species groups.

A new technique, RAPD-PCR (Williams et al., 1991), recently adapted for use with aphids (Black et al., 1992) was used in the molecular studies undertaken. Using PCR and single small primers, the simple technique does not require live insects. Each primer sequence yields a new banding pattern. The patterns are quite distinct between species, and often a collection of primers can be found empirically that will distinguish among geographical variants of the same species. Finding the distinguishing markers can be difficult; but once found, the diagnostic assay is relatively quick and easy.

For predatory ladybeetle, *Coccinella septempunctata,* a set of four different primers (20 primers were tried) appears to separate five collections from USA (Delaware), France, Syria, Ukraine, and Moldavia. A sample of *C. transversogutta biinterrupta* displayed small differences from *C. septempunctata* with almost every primer tested. *Hippodamia variegata* samples from Canada, France, Morocco, Moldavia, Kirghizia, and Chile appear to be separated with four primers, although the analysis is a bit more complicated. For *Propylea quatuordecimpunctata* several primers separate Canada from France and USSR. The latter two are very similar. One primer looks as if it might distinguish all three.

In the parasitic Hymenoptera, eight different samples of *Diaeretiella rapae* were received from the Stillwater collection (France (2), Greece (2), Jordan (1), Pakistan (2), and Syria (1)). Separation could be obtained for the Jordanian, one of the French, the Syrian, and one of the Greek lines. The other Greek sample is probably separable, but the second French and the two Pakistani lines have exhibited excessive individual variability and overlap of banding patterns. Four additional lines have been received from APHIS but not fully examined. Three strains of *Aphidius matricariae* were examined, and initial tests suggest that the USA sample is distinct but the Iraq and USSR samples are not (24 primers tried). Finally, single samples of *Aphidius picipes, Aphidius colemani,* and *Lysiphlebus testaceipes* were briefly examined. It seems to be relatively easy to distinguish all five species. The problem of strains not being distinct may reflect the reality of nature, but,more likely is the result of inadvertent mixing of colonies during laboratory rearing. The three or four *D. rapae* lines that have been most similar all were reared at Stillwater. The two *A. matricariae* lines that have resisted separation came from Mission, Texas. The problem of genetic mixing of laboratory-reared lines has been encountered before with screwworm flies and boll weevils.

Other approaches were also employed in the effort to find DNA markers. Standard analysis of mitochondrial restriction fragment patterns did not work with *D. rapae* because the individual insects are too small. This technique might be useful for the coccinellids or other larger predators, but was not tried. A region spanning the 12S and 16S mitochondrial RNA genes was amplified for several individuals of each of the four species of coccinellids using PCR. The PCR product was cut with eight different restriction enzymes and fragment patterns compared. No differences were found in the intraspecific patterns. Nucleotide sequencing, which would provide much more information from the same piece of DNA, was not tried. The interspecific patterns were quite distinct (*C. septempunctata* and *C. transoversogutta* were very similar) and might be a useful tool for biosystematics studies. This type of PCR should be feasible with the parasites as well.

## Biosystematics

**Mission:** To provide identifications and verifications for RWA and its natural enemies.

**Systematic Entomology Laboratory (SEL)**
**Beltsville, MD**
**M. B. Stoetzel**

During FY91, Dr. Stoetzel continued to provide identifications and verifications for *Diuraphis noxia* (Mordvilko). There is a continuing need for voucher specimens to be submitted to the SEL as researchers search for parasites and predators of the RWA.

From April 3-7, 1991, Dr. Stoetzel worked with Dr. Rebeca Peña de Garcia, ENCB-IPAI, Mexico, to collect specimens of *Diuraphis noxia* (Mordvilko), the RWA, and *D. mexicana* (J. M. Baker). These specimens are being used in Stoetzel's study of the species in the genus *Diuraphis*.

During much of FY91, Dr. Stoetzel had little or no technical support, which delayed progress on her revision of the genus *Diuraphis,* now expected to completed during FY92.

## Simulation Modeling

**Mission:** To develop quantitative technology for incorporation into management decision support systems.

**Plant Science Research Laboratory (PSRL)**
**Stillwater, OK**
**N. C. Elliott**

A study is being conducted in cooperation with G. L. Hein (University of Nebraska) on the field population dynamics of the RWA in western Nebraska. The purpose of the study is to determine, quantify, and model processes of reproduction, mortality, morph determination, and movement (emigration and immigration) in wheat fields and CRP grasslands in order to obtain a more detailed understanding of the life system of the pest, and therefore to develop better strategies to control its populations. Detailed data were collected during 1991 in both winter wheat and CRP fields. Progress toward our objective was limited somewhat by the generally low RWA populations that occured throughout most of the Great Plains in 1991.

## Biological Control

**Mission:** To collect, study, and release exotic natural enemies of the RWA into the RWA-infested grain growing regions of the United States as a control strategy. To develop IPM strategies for RWA management that will reduce the quantity of pesticides presently used in RWA control.

**Plant Science Research Laboratory (PSRL)**
**Stillwater, OK**
**D. K. Reed, N. C. Elliott**

The new building for biological control research has been completed. Five rearing rooms with controlled temperature and lighting, used now for rearing of several predator and parasitoid species, increase the overall research capabilities of the unit. The laboratory and work space are adequate for conducting much of the laboratory research, and contribute toward getting the field research done.

Tritrophic Interactions

Research on tritrophic interactions among various RWA host plants, the aphid, and natural enemies are continuing. One report concerning effect of various small grain entries has been published (Reed et al., 1991). Another report of research on grass entries is currently in review at a major journal. Research is nearly complete on effect of long-term association of parasitoids with various host plants, and a study of effects of host plants on fungal pathogens has been initiated in cooperation with T. J. Poprawski (USDA-ARS. PPRL). A field tritrophic study to attempt to confirm laboratory research was initiated this fall, to be completed in the spring of 1992. Results and conclusions of these studies so far indicate the importance of consideration of tritrophic interactions by plant breeders as they develop resistant lines of plants.

A greenhouse tritrophic study was conducted in cooperation with a Doctoral candidate, R. K. Campbell, and his major advisor, R. D. Eikenbary (Oklahoma State University). This study included not only host-plant cultivar effect, but also effect of drought on parasitoids on RWA. Both parameters affected parasitoid growth and development.

Field Release Studies

Natural enemies of RWA were released in Oklahoma, Texas, Nebraska, and Colorado in the spring of 1991. Releases were made of two parasitoids (*Aphelinus asychis* and *Aphidius colmani*) and two coccinellid predators (*Hippodamia variegata* and *Coccinellina ancoralis*) from South America. These natural enemies of aphids were collected in November 1990 by D. K. Reed and K. Pike (Washington State University). Another parasitoid (*Aphidius matricariae*) from USSR was also released. Both open-field and screen-caged releases were made in all areas. Recoveries of several of the species released were made from the

caged release areas in all four states, indicating the possibility of establishment. Low aphid populations within the field prevented confirmation of spread of the natural enemies. The study showed that caged releases may be more effective than open-field releases within the cereal crops ecosystem, allowing more time for the natural enemies to build up in the absence of detrimental organisms prior to exposure. Cooperators on this project included G. L. Hein (Nebraska), M. A. Karner (Oklahoma), G. J. Michels (Texas), and C. B. Walker (Colorado).

Studies have been initiated on the basic biology and natural history of RWA predators. This information will aid in prioritizing predators for field releases. Current research is on the laboratory biology and life history of the coccinellids *Cycloneda ancoralis* and *Propylea quatuordecimpunctata*.

Natural Enemy Evaluations

Studies are being conducted to evaluate RWA natural enemies for their potential to establish populations in wheat agroecosystems in the Great Plains. Two factors that are critical for parasitoids are the ability to successfully overwinter, and the availability of suitable alternate hosts during portions of the growing season when RWA are unavailable as hosts. In cooperation with J. R. Nechols (Kansas State University) and R. W. Kieckhefer (USDA-ARS, NGIRL), field studies were initiated on the overwintering ability of RWA parasitoids in the Great Plains. *Diaeretiella rapae* and *Aphidius matricariae* overwintered successfully in field cages in Kansas and Oklahoma but did not survive the winter in South Dakota. Laboratory and field studies are also being conducted to determine the range of alternate host occurring on crop and non-crop vegetation that can be utilized by parasitoids imported for RWA biological control.

A visiting scientist, H. C. Reed from Oral Roberts University, Tulsa, Oklahoma, completed a cooperative research project on reproductive and life table statistics of *Diaeretiella rapae* and *Aphidius matricariae* on RWA. He found that adult longevity of *A. matricariae* females was longer but *D. rapae* females began ovipositing sooner due to a decreased immature period. Also, mean reproductive capacity of *D. rapae* was higher than *A. matricariae*. Sex ratio of progeny was more biased toward females in *D. rapae*. The jackknife estimate of the intrinsic rate of increase ($r_m$) for *D. rapae* was higher, but with more variance, than the $r_m$ value for *A. matricariae*. Overall, the study indicates that *D. rapae* may be a more effective parasitoid than *A. matricariae* based on conditions of the experiment.

**Beneficial Insects Introduction Research Laboratory (BIIRL)**
**Newark, DE**
**L. R. Ertle, P. W. Schaefer**

1991 Quarantine Activities - *Diuraphis Noxia* (Mordvilko)

A total of 23 consignments of potential natural enemies of RWA were received at BIIRL in 1991. Of the total 3,657 specimens shipped, 3,162 were received alive. The material was collected in the USSR (Kazakstan and Tashkent), Greece, France, Italy, Syria, and Canada. Only six of the consignments were coccinellids; no "new" beneficial species were recovered from these shipments. However, *Coleomegilla quadrifasciata* (S.) (ex: 1990) and *Coccenellina ancoralis* Guerin (ex: 1990 and 1991) identifications were confirmed, and they were released from quarantine. Two consignments of Syrphid predators belonging to the genera *Episyrphis* and *Sphaerophoria* collected in Greece and Italy represented a new group of potential RWA predators. Nine different species were included in these two shipments, but culture attempts failed due to inexperience, identification problems, and poor female/male sex ratios and associations. Five consignments of *Leucopis* (*Ninae, Atritarsis,* and sp.) were received from Canada and USSR and transhipped to APHIS, Niles, Michigan, for further studies. Ten consignments of Braconid parasites were processed from France, USSR, and Syria. A "new" (different) species of *Lysiphlebus* was recovered from the USSR, but only 8 (1 female) of the 100 mummies emerged; the culture attempt in quarantine failed.

Thirty-four shipments (6,119 specimens) of beneficial parasites and predators comprising 10 different species were released from quarantine. Most of the predators were sent to R. L. Flanders, APHIS, Niles, Michigan for evaluation. All the parasites and samples of the South American coccinellid species were sent to D. K. Reed, USDA-ARS, PSRL.

At year end, BIIRL continues to maintain (on *Acyrthosiphon pisum*) cultures of *Anatis labiculata* (Say), *Adalia bipunctata* (L.) (3 biotypes), *Coccinella septempunctata* (L.), *Coccinula quatuordecimpustulata* (L.), *Eriopis connexa* Germar, *Hippodamia tredecimpunctata* (L.) (2 biotypes), *H. variegata* (Goeze) (2 biotypes), *Hippodamia* sp. (3 biotypes), *Propylea quatuordecimpunctata* (L.) (5 biotypes), *Scymnus frontalis* F., and *Semiadalia undecimnotata* (Schneider).

**European Biological Control Laboratory (EBCL)**
**Montpellier, France**
**K. R. Hopper**

Collection/Shipment

On trips to southern France, Balkans (Yugoslavia and Greece), Caucasus and Central Asia (USSR), we collected large numbers of natural enemies of *D. noxia* in the following species and groups: parasitoids - *Aphelinus varipes, Aphelinus asychis, Aphelinus* sp. near *varipes, Aphidius* spp., *Diaeretiella rapae*; predators - Syrphidae, Coccinellidae, and Chamaemyidae, and pathogens - *Pandora neoaphidis*. Over 8000 parasitoids in at least six species and over 700 predators in the families Chamaemyiidae, Syrphidae, and Coccinellidae (Table 1) were shipped to the United States. In many cases, we cannot give species identifications, because we sent all the material as mummies or pupae to quarantines in the United States and we have not yet received information of the identity of the insects that emerged.

EBCL staff received, reared, and shipped natural enemies collected by S. Halbert (University of Idaho) and T. Poprawski (USDA-ARS, BIIRL) in the USSR (Kazakstan and Kirgizia) and by R. Miller (ICARDA, Syria) in Syria. Drs. Halbert and Poprawski were also provided transportation during their visit to EBCL, and with material and equipment for their expedition.

We have colonies at EBCL of *A. varipes* from Montpellier, *A. asychis* from Antibes and Montpellier in France, and *Aphelinus* sp. near *varipes* from the northern Caucasus, Uzbekistan, and Kazakstan.

Limitation of *D. noxia* Populations in Southern France

We conducted a field cage experiment on the impact of natural enemies on *D. noxia* in the Montpellier area and found that exclusion of predators and parasitoids led to an 8-fold increase in density. We eliminated the effects of the cages on microclimate and on immigration by using open-top control cages and measuring the frequency of alates. We plan to repeat this experiment for a third season and to expand it to include monthly estimates of parasitism and the impact of predators and parasitoids by exposing caged and uncaged potted plants with aphids.

R. Dabire, A. Nobrega-Farias, C. Lesieux, and J. Vidal, students from local universities, conducted research on the impact of coccinellids and aphelinids on *D. noxia* abundance, on host species preferences of coccinellids, on host stage preferences of aphelinids, and on the effects of wheat variety and irrigation on *D. noxia* and damage. *Coccinella septempunctata* could account for as much as half of the losses of *D. noxia* on uncaged populations in the field. *C. septempunctata* fourth instar larvae and adults

Table 1. Shipments to the United States in 1991 of predators and parasitoids of *Diuraphis noxia*.

| File No. EPL 91 - | Organism | Number | Location Collected |
|---|---|---|---|
| 01 | *Aphelinus varipes* | 500 | Montpellier, France |
| 04 | *A. varipes* | 1000 | Antibes, France |
| * 05 | *A. asychis* | 172 | Antibes, France |
| 08 | *Aphelinus* sp, | 53 | Dmitrievka, Kazakstan |
| | *Aphidius* sp. | ? | |
| | *Leucopis* sp. | ? | |
| | Syrphidae | ? | |
| 09 | *Aphelinus* sp. | 62 | Dmitrievka, Kazakstan |
| 10 | *Coccinella septempunctata* | ? | Kirgizia & Kazakstan |
| | Coccinellidae | ? | |
| 11 | *C. septempunctata* | 159 | Thermi, Greece |
| 12 | Syrphidae | 203 | Thermi, Greece |
| 13 | *C. septempunctata* | 102 | Southeast of France |
| 14 | *P. quatuordecimpunctata* | 28 | Southeast of France |
| 15 | *Adonia variegata* | 176 | Southeast of France |
| 16 | *C. septempunctata* | 35 | Sicilia, Italy |
| 17 | Syrphidae | 15 | Sicilia, Italy |
| * 23 | *A. asychis* | 104 | Antibes, France |
| 24 | *A. asychis* | 100 | Dmitrievka, Kazakstan |
| 25 | *A. asychis* | 300 | Dmitrievka, Kazakstan |
| 26 | *Aphidius matricariae* | 100 | Alma-Ata, Kazakstan |
| 27 | *Lysiphlebus fritzmuelli* | 100 | Alma-Ata, Kazakstan |
| 28 | *Aphelinus*sp. | 200 | Dmitrievka, Kazakstan |
| 29 | *Diaeretiella rapae* | 100 | Tel Hadza, Syria |
| 30 | *A. asychis* | 221 | Dmitrievka, Kazakstan |
| 31 | *A. matricariae* | 100 | Alma-Ata, Kazakstan |
| 32 | *L. fritzmuelli* | 100 | Alma-Ata, Kazakstan |
| 33 | *Aphelinus* sp. | 100 | Dmitrievka, Kazakstan |
| 56 | *Leucopis* sp. | 50 | Tashkent, Uzbekskaja |
| 57 | *Aphelinus* sp. | 300 | Budennovsk, Rossijskaja |
| 58 | *A. varipes* | 500 | Dmitrievka, Kazakstan |
| 59 | *Aphelinus* sp. | 300 | Budennovsk, Rossijskaja |
| 60 | *A. varipes* | 500 | Dmitrievka, Kazakstan |
| 61 | *D. rapae* | 200 | Larissa, Greece |
| 62 | *D. rapae* | 500 | Dmitrievka, Kazakstan |
| 63 | *D. rapae* | 200 | Larissa, Greece |
| 64 | *D. rapae* | 500 | Dmitrievka, Kazakstan |
| 65 | *D. rapae* | 200 | Dmitrievka, Kazakstan |
| 66 | *D. rapae* | 200 | Dmitrievka, Kazakstan |
| 67 | *A.* near *varipes* | 200 | Tashkent, Uzbekskaja |
| 68 | *A.* near *varaipes* | 200 | Tashkent, Uzbekskaja |
| 69 | *A. asychis* | 190 | Grabels, France |
| * 70 | *A. asychis* | 32 | Antibes, France |
| * 71 | *D. rapae* | 100 | Tashkent, Uzbekskaja |
| 72 | *D. rapae* | 200 | Tashkent, Uzbekskaja |
| * 76 | *A.* near *varipes* | 500 | Pyatigorsk, Rossijskaja |
| * 77 | *A.* near *varipes* | 50 | Pyatigorsk, Rossijskaja |
| 78 | *A.* near *varipes* | 500 | Pyatigorsk, Rossijskaja |

* Form AD-941 received from Newark

were capable of preying on *D. noxia* third and fourth instar nymphs in folded leaves, although the level of predation was reduced, and fed and starved beetles did not show significant preferences among five species of cereal aphids. The experiment on the impact of aphelinids will have to be repeated because of poor establishment of the parasitoids in field cages. In the laboratory, *Aphelinus asychis* showed no significant preferences in parasitization among first through fourth instar nymphs of *D. noxia* and predation by *A. asychis*. Irrigation and cultivar had little effect on the density of *D. noxia* or damage symptoms. However, irrigation did reduce the level of damage qualitatively.

Population Biology of Collection and Introduction of Predators and Parasitoids

Mass-rearing colonies with aphelinid parasitoids of *D. noxia* collected in France, Russia, Uzbekistan, and Kazakstan, and isofemale lines collected in the Montpellier area were established. Mass-rearing colonies and isofemale lines of *A. varipes* and *A. asychis* collected in previous years from Antibes and Montpellier, France, were continued. Using this material, an experiment on heritability of search rate in *A. asychis* was conducted. Comparisons among lines and parent-offspring regression, should that there was significant variation and that search rate tended to be inheritable. However, we did not measure enough progeny per line, and we plan to redo this study with an improved design using a diallel cross and measuring more characters. Cooperative work was begun with A. Le Ralec (ENSAM, Montpellier) on morphological and physiological differences between parasitoids from different aphid species. Colonies of *A. varipes* from *D. noxia* and *Rhopalosiphum padi* were established to test whether they are genetically different.

Cooperative work with R. Roush (Cornell University) was continued on strategies for avoiding genetic change during laboratory rearing for biological control introductions, and a paper on the use of isofemale lines to avoid laboratory adaptation was written. Hopper, Roush, and W. Powell (Rothamsted Experimental Station) reviewed the literature on genetic variation in natural enemies (particularly parasitoids) and will submit a paper on this topic to the Annual Review of Entomology.

Hopper presented a paper on "Allee Effect in Biological Control" at the 6th European Workshop on Insect Parasitoids in Perugia, Italy (3-5 April). This paper dealt with mathematical models of natural enemy colonization designed to test whether low densities, and thus failure to find mates, that result from dispersal after introduction could account for failure of some biological control agents to establish.

Staff from EBCL, IIBC, and CSIRO met at Silwood Park, England, (21-23 January) to discuss cooperation on insect biological

control projects. This meeting resulted in a coordination of exploration in Yugoslavia and Greece by EPL and IIBC, a cooperative trip to the USSR by K. Carl (IIBC) and K. R. Hopper (EBCL), and a proposal for cooperative experiments by IIBC and EBCL in Greece and/or Yogoslavia on the factors that limit *Diuraphis noxia* abundance.

R. Burton and N. Elliott (USDA-ARS, PSRL) and F. Peairs (Colorado State University) visited the laboratory in September to discuss the *Diuraphis noxia* program.

**Plant Protection Research Laboratory (PPRL)**
**Ithaca, NY**
**T. J. Poprawski, S. P. Wraight, N. L. Underwood**

Natural Prevalence of Endemic Fungal Pathogens, Hymenopteran Parasitoids, and Dipteran Predators in Populations of Cereal Aphids in Spring-Planted Grains in Colorado (with W. Meyer and F. Peairs, Dept. of Entomology, Colorado State University, Fort Collins).

The state of Colorado has experienced some of the highest levels of RWA damage in the United States (1989 losses estimated at over 20 million dollars) and is considered an important site for releases of biocontrol agents under the USDA-ARS RWA Biological Control Program. In June 1990, field surveys were initiated to provide baseline information on the identity and prevalence of endemic pathogens, parasitoids, and predators and to assess their relative impacts on populations of *Diuraphis noxia* and associated cereal aphids. Sampling was conducted primarily in two areas of the state, near Fort Collins/Laporte/Wellington (Northern Plains Area) and near Akron (Central Great Plains Area). The sampled fields were spring-planted wheat and barley, smaller than 3 hectares, and were not treated with insecticides. All were dry-land cultures except for those in Wellington, which were maintained under intensive irrigation provided by center-pivot sprinkler systems.

The RWA was the most abundant aphid at all sample sites. *Rhopalosiphum padi*, the bird cherry oat aphid, was the only other cereal aphid collected in significant numbers during the season. Aphid populations were greater in nonirrigated fields than in irrigated fields.

Random tiller samples indicated that parasitoid prevalence was low (less than 5%) throughout the season. Only 55 mummified aphids were found in the samples collected from all fields during June; among these were 53 *D. noxia*, 1 *R. padi*, and 1 *Schizaphis graminum*. All appeared to be of the aphidiid type. Emergence

from these mummies (all *D. noxia*), produced 13 *Diaeretiella rapae* and 8 charipid and 2 pteromalid hyperparasites. During July, 133 mummies were collected. Of these, 114 (106 *D. noxia,* 5 *R. padi*, and 3 *S. graminum*) were aphidiid types. Emergence yielded 23 *D. rapae*, 10 pteromalid and 2 charipid wasps (all from *D. noxia* except 1 charipid from *R. padi*). The other 19 mummies (12 *D. noxia* and 7 *R. padi*) were aphelinid types. All were found in late season samples from dry-land fields at Laporte and Fort Collins. Emergence produced 8 *Aphelinus* primary parasites, 1 pteromalid hyperparasite from *D. noxia,* and 1 charipid and 2 encyrtid hyperpaarasites from *R. padi*.

The only predators consistently found feeding on aphids within *D. noxia* damaged (rolled) leaves were dipteran larvae. Larvae of the family Syrphidae were the dominant predators, occurring at all sites but one. Populations were low, however usually less than 0.1 larvae per tiller through June and only 0.2-0.3 larvae per tiller by the end of the season. A few larvae of the family Chamaemyiidae were also found.

Aphid-pathogenic fungi were active at nearly all sample sites; however, prevalence in nonirrigated fields did not exceed 2.5%. Three species were identified: *Entomophthora chromaphidis, Pandora neoaphidis*, and *Conidiobolus obscurus*. In irrigated fields, *E. chromaphidis* was the dominant pathogen earlier in the season, with peak prevalence of 13% recorded on 22 June. *Pandora neoaphidis* was not detected until late June, but it rapidly reached epizootic levels (44% infection) by 18 July. Highest prevalence of *C. obscurus* (20%) coincided with that of *P. neoaphidis*.

Baseline Characterization of the Endemic RWA Pathogen-Parasite Complex in Southwestern Idaho (with S. E. Halbert and T. M. Mowry, University of Idaho, Parma)
The finding in 1990 of only minimal activity of aphid pathogenic fungi in commercial dry-land wheat and barley fields in Colorado resulted in the focussing of our research effort on irrigated production systems. The 1991 field research was conducted at the SW Idaho Research and Extension Center of the Univerisity of Idaho at Parma.

Despite outbreak levels in 1990, RWA populations were extremely low in the spring of 1991, presumably due to a cold winter and a cool, wet spring. No economic damage was reported in either fall- or spring-planted grains in the region. Sampling of a fall-planted wheat research plot revealed considerable parasitoid activity by 21 May. At that time, 13.4% of late-instar and adult *Diuraphis noxia* were parasitized by *Diaeretiella rapae*. Parasitoid prevalence increased markedly over the subsequent 7 days to 52% and peaked at 76.1% by 11 June. The aphid population increased from nearly undetectable levels on 10 May to only 0.48 aphids per

tiller by 8 June. It was apparent that the few isolated colonies of *D. noxia* that survived the winter and increased very slowly during the cool spring were effectively targeted by the aphidiid parasite.

The *D. noxia* population in spring-planted grain also developed slowly, not attaining a density of one aphid per tiller until 21 June. Parasite prevalence remained low (<14%) through 25 June, but increased sharply to 37% on 28 June. In contrast to the continued increase to very high levels of parasitism observed in the winter grain, however, prevalence dropped to only 25% by the next sample date (2 July) and then remained at that level until sampling was terminated 5 July. The approximate doubling of the RWA population under weather conditions more favorable than existed during sampling of the winter wheat and the rapid increase of other cereal aphids attractive to *D. rapae* may account for the stabilization of the rate of parasitism of *D. noxia* during this period.

By late June, the spring-planted grain had become heavily infested with *Metopolophium dirhodum*, the rose-grass aphid, and on 2 July several individuals of this species were found infected with the entomophthoralean fungus *Pandora neoaphidis*. Within 3 days, the fungus had spread throughout the field, infecting 8% of the population (which was increasing rapidly and had reached a density of 18 per tiller). At the same time, no infections were observed in the lower density *D. noxia* population (2.4 per tiller). No additional random sampling was conducted, because the grain was rapidly maturing. However, samples of heavily damaged/infested tillers collected 10 July revealed 2.3% infection of the RWA.

Since the low aphid populations in the fall- and spring-planted crops supported little fungal activity, study of these natural control agents was necessarily undertaken in a late-planted experimental wheat plot (such plantings are commercially infeasible due to weed and insect pest problems). The plot was planted 15 May, and sampling was initiated 28 May. The first RWA were detected 7 June. The population remained low (<0.3 per tiller) until late June and then, with improving weather conditions, increased rapidly to over 15 per tiller by 13 July. At the same time, populations of associated cereal aphids increased to extremely high levels (150 per tiller). The dominant species was *M. dirhodum*, followed in order by *Rhopalosiphum padi, Schizaphis graminum, Sitobion avenae*, and *Sipha elegans*. Sampling results suggest that *M. dirhodum* individuals infected with *P. neoaphidis* migrated into the plot in early July, and the combination of high host density and moist conditions created by the sprinkler irrigation system triggered a large scale epizootic. Infection of *M. dirhodum* increased from 3.1% on 5 July to 79% on 16 July and then remained high, peaking at 89.5% on 23 July.

The population declined 99.3% from the 13 July peak levels of 88.9 aphids per tiller. Infection of *D. noxia* increased from 3.5% on 5 July to a maximum of 80% on 16 July, but then declined to 35% over the next 3 days and remained at that approximate level until termination of sampling on 26 July. During this period, the population declined 81% from the peak density of 21 aphids per tiller recorded 16 July. The percentage of tillers infested with *D. noxia* also declined from 75 to 42%.

It is probable that the higher level of infection of *M. dirhodum* compared with *D. noxia* is a consequence of microhabitat differences. *M. dirhodum* inhabits the open leaf surface while *D. noxia* lives within rolled leaves of the damaged tillers. The approximately 20% of *D. noxia* that survived the peak period of fungal transmission may have inhabited secluded areas where the fungal inoculum (spores) did not penetrate.

Data relating the population responses of other cereal aphid species to the fungal epizootic are not yet analyzed. During the course of the epizootic, two other species of aphid-pathogenic fungi, *Entomophthora chromaphidis* and *Conidiobolus thromboides*, were found; however, prevalence of these pathogens never exceeded 1%. Rates of parasitism of RWA by *D. rapae* in the late-planted wheat were similar to those observed in the earlier planting , being approximately 10% through 27 June, then rising abruptly to 33% by 2 July and to 38% by 5 July. Subsequently, however, parasotoid prevalence decreased to 20% by 16 July, the date corresponding to the peak of the fungal epizootic. This coincidence of high fungal and low parasitoid prevalence is likely a reflection of a negative interaction between the two natural control agents. Our laboratory study indicates that parasitoid larvae are usually not able to complete development following fungal infection, as the fungus normally kills the host within 3-4 days at moderate temperatures.

It is noteworthy that the levels of parasitism observed in this study are substantially higher than the 0-5% rates we reported from Colorado last year. The reason for the apparently greater success of cereal aphid parasitoids in Idaho is unknown.

The results of this study indicate the great importance of entomopathogenic fungi as natural regulators of cereal aphid populations. Many studies have described equally decimating disease outbreaks. Unfortunately, under normal weather conditions, these epizootics generally occur late in the season in high-density host populations that have already severely damaged the crop. Such epizootics probably reduce numbers of oversummering aphids and thus pest pressure on fall-planted grains. However, if fall and winter weather conditions are favorable, severely damaging aphid outbreaks will still occur in the spring. Research is therefore needed to assess the potential

impact of fungi on these early-season aphid populations.

We recognize the need for research addressing four fundamental problems.

1. Control of early-season aphid populations will require introduction of pathogens that are transmitted most efficiently at temperatures of 20-25° C into aphid populations developing under cool springtime weather conditions. To address this problem we plan to screen or select for pathogen isolates with below normal temperature optima.

2. A second problem involves delivery of fungal inoculum into *D. noxia* colonies developing within the tightly rolled leaves of the infested tillers. In the above-described natural epizootic, this apparently occurred via the spread of inoculum from the fungus being rapidly transmitted and augmented in the *M. dirhodum* population inhabiting the open leaf surfaces. The hymenopteran parasitoids may have been important fungus vectors in this instance, and also the greenbug (*S. graminum*) that inhabits both open and rolled leaves. We propose to achieve this experimentally through three possible means: application of dry-formulated or fresh fungal mycelium or conidia, release of infected aphids, or release of fungus-inoculated hymenopteran parasites.

3. The third problem area involves the dependence of entomopathogenic fungi on high moisture conditions for sporogenesis, spore germination, and host infection. Irrigated cropping affords the opportunity to manipulate environmental moisture conditions to favor disease development. Studies are needed to determine the optimal irrigation schedule for transmission of aphid-pathogenic fungi (but minimizing spread of plant-pathogenic species) within the economic framework of the target crop production system.

4. Finally, the fact that entomopathogenic fungi are potentially important antagonists of parasitoids and predators competing for the same host calls for detailed study of host-pathogen parasitoid-predator interactions. Introductions of fungal pathogens into the field must include assessment of impact on the indigenous or experimentally established exotic parasite/predator complexes. Biological control strategies must be developed that enhance the integrated operation of these major groups of aphid control agents.

Foreign Exploration

T. J. Poprawski and S. E. Halbert (University of Idaho) travelled in Soviet Central Asia from 26 April-18 May 1991 on an expedition to find natural enemies for the RWA. RWA was very scarce this spring due to the hard winter and possibly to the outbreak in 1990. However the aphid was found to be abundant in a few fields that were typical *D. noxia* habitat--sparse but healthy plants.

Dipteran predators and/or their eggs were associated with *D. noxia* wherever RWA was found. Aphidiidae mummies were found in RWA colonies in Kirghizia, and Aphelinidae mummies were found in RWA colonies in Kazakstan.

Other cereal aphids found in Central Asia on wheat and barley included *Sitobion avenae, Schizaphis graminum, Schizaphis* sp., *Metopolophium dirhodum, Rhopalosiphum padi, R. maidis, Sitobion* near*fragariae, Forda marginata,* and *Atheroides* sp.

Our collecting netted about 75 dipteran predators (at least two species of Syrphidae and one including *A. asychis* and *A.* near *varipes*), 5-10 Braconidae mummies (species not yet determined), several RWA infected with fungal pathogens, 530 *Coccinella septempunctata*, and several hundred other Coccinellidae. Only biomaterial that survived handling under the difficult conditions of the trip is accounted for here. We are confident that at least some of the natural enemies we found will feed on *D. noxia* at extremely low densities. Some may also have a very limited host range, as they were not found associated with other aphids.

Numbers shipped to the U.S. as of 30 August 1991
(communicated by D. Coutinot, EBCL, Montpellier, France)
*Aphelinus* spp. 53 mummies to Texas A&M
*Aphidius* spp. 05 mummies to Texas A&M
Chamaemyiidae + Syrphidae 200 larvae to Texas A&M
*Aphelinus* spp. 62 mummies to USDA-ARS, BIIRL
*Coccinella septempunctata* 530 adults to USDA-ARS, BIIRL
*Adonia, Hippidamia, Psyllobora, Subcoccinella* spp. 300 adults to USDA-ARS, BIIRL
*Aphelinus asychis* 100 F1 mummies to USDA-ARS, BIIRL
*A. Asychis* 300 F1 mummies to USDA-ARS, BIIRL
*Aphidius matricariae* 350 F2 mummies to USDA-ARS, BIIRL
*Lysiphlebus fritzmuelli* 420 F2 mummies to USDA-ARS, BIIRL
*Aphelinus* sp. 500 F1 mummies to USDA-ARS, BIIRL

*A. asychis* 221 F1 mummies to Texas A&M
*A. matricariae* 280 F2 mummies to Texas A&M
*L. fritzmuelli* 460 F2 mummies to Texas A&M
*Aphelinus* spp. 500 F1 mummies to Texas A&M
*Aphelinus* near *varipes* 500 F1 adults to Texas A&M
*A.* near *varipes* 500 F2 adults to USDA-ARS, BIIRL
*Diaeretiella rapae* 500 F2 adults to USDA-ARS, BIIRL
*D. rapae* 500 F3 mummies to USDA-ARS, BIIRL
*D. rapae* 500 F2 adults to Texas A&M
*D. rapae* F3 mummies to Texas A&M
*Beauveria bassiana, Conidiobolus* spp., *Pandora neoaphidis* and *Zoophthora radicans* isolates from RWA hand-carried to USDA-ARS,PPRL.

A third species of *Aphelinus* (close to but not *varipes*) is being identified by French taxonomists. It might represent a new species. It does very well on RWA and is maintained in culture at the EBCL for future shipment.

Cultures of *A. asychis, A.* near *varipes, D. rapae, A. matricariae,* and *L. fritzmuelli* are also maintained at the EBCL for host acceptance studies and future shipments to U.S. cooperators. All these species of parasitoids do very well on RWA.

Several species of parasites of Diptera collected during the expedition have already been mass-reared (Texas A&M and APHIS, Niles, Michigan) and released in Idaho, Washington, Texas, and several other U.S. states. The fate of coccinellids is unknown to us at the time of writing.

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